

FIG. 1A

09060872.041598

365T40" 22809060

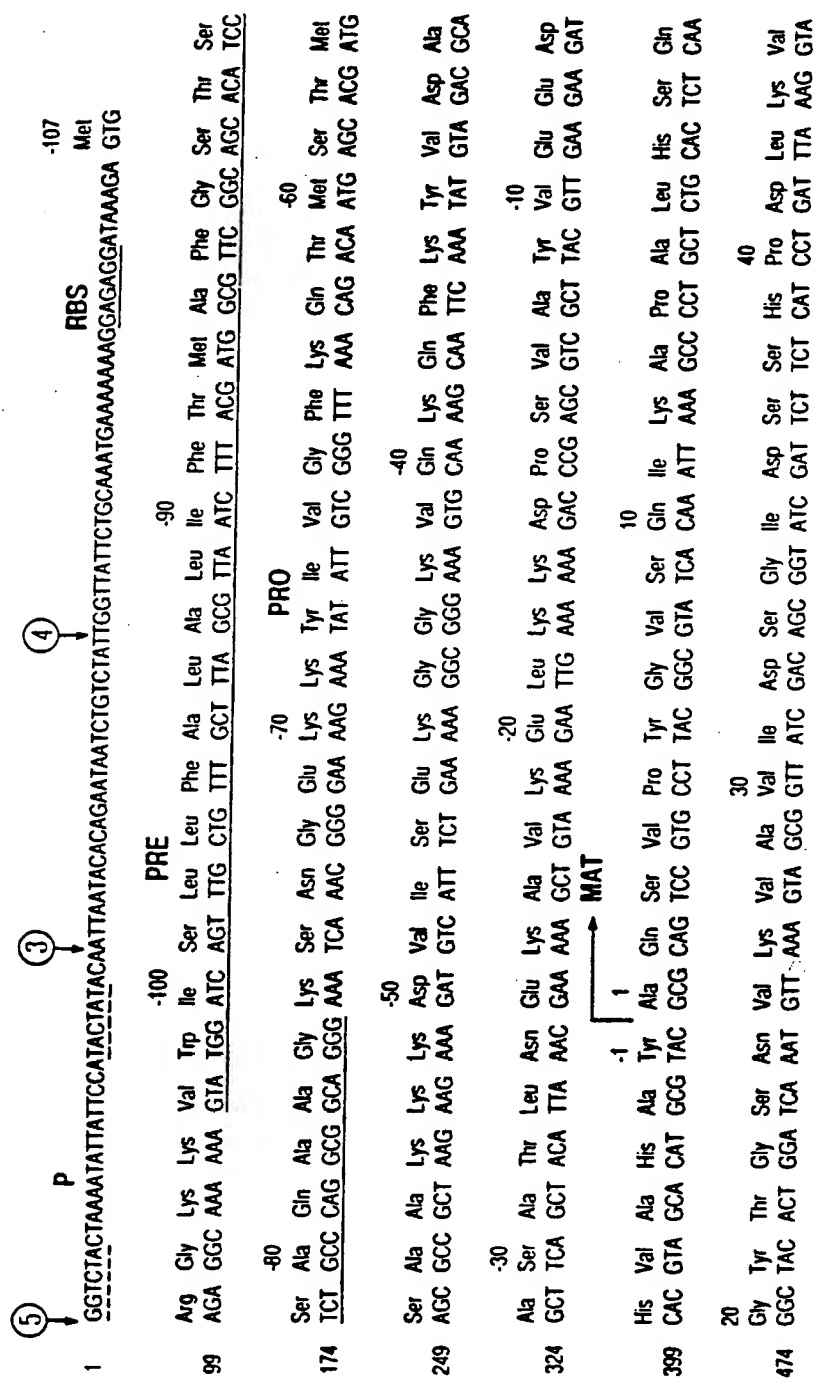


FIG. 1B - 1

B65F40" 22809060

549 Ala Gly Gly Ala Ser Met Val Pro Ser Gly Thr Asn Pro Phe Gln Asp Asn Asp 60 Asp
 GCA GGC GGA GCC AGC ATG GTT CCT TCT GAA ACA AAT CCT TTC CAA GAC AAC AAC TCT CAC GGA ACT CAC GTT GCC
 70
 524 Thr Val Ala Ala Leu Asn Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Ser Ala 90
 GGC ACA GTT GCG GCT CTT AAT AAC TCA ATC GGT GTA TTA GGC GTT GCG CCA AGC GCA TCA CTT TAC Gly Tyr Ala Val Lys
 80
 699 Val Leu Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn Asn Met
 GTT CTC GGT GCT GAC GGT TCC GGC CAA TAC AGC TGG ATC ATT AAC GGA ATC GAG TGG GCG ATC GCA AAC AAT ATG
 100
 120 Asp Ala 100
 774 Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Val Asp Lys Ala Val Ala
 GAC GTT ATT AAC ATG AGC CTC GGC GGA CCT TCT GGT TCT GCT TTA AAA GCG GCA GTT GAT AAA GCC GTT GCA
 130
 849 Ser Gly Val Val Val Val Val Ala Ala Gly Asn Gly Thr Ser Gly Thr Ser Ser Ser Thr Val Gly Tyr Pro Gly
 TCC GGC GTC GTA GTC GTT GCG GCA GCC GGT AAC GAA GGC ACT TCC GGC AGC TCA AGC ACA GTG GGC TAC CCT GGT
 150
 170 Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro
 AAA TAC CCT TCT GTC ATT GCA GTA GGC GCT GTT GAC AGC AGC AAC CAA AGA GCA TCT TTC TCA AGC GTA GGA CCT
 180
 924 Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly
 GAG CTT GAT GTC ATG GCA CCT GGC GTA TCT ATC CAA AGC ACG CTT CCT GGA AAC AAA TAC GCG GCG TAC AAC GGT
 200
 999 Thr Ser Met Ala Ser Pro His Val Val Ala Gly Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr
 220
 1074 ACG TCA ATG GCA TCT CCG CAC GTT GCC GGA GCG GCT GCT TTG ATT CTT TCT AAG CAC CCG AAC TGG ACA AAC ACT
 240

FIG. 1B - 2

1149	Gln	Val	Arg	Ser	Ser	Leu	Glu	Asn	Thr	Thr	Thr	Lys	Leu	Gly	Asp	Ser	Phe	Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn
	CAA	GTC	CGC	AGC	AGT	TTA	GAA	AAC	ACC	ACT	ACA	AAA	CTT	GGT	GAT	TCI	TTC	TAC	TAT	GGA	AAA	GGG	CTG	ATC	AAC
270																									
1224	GTA	CAG	GCG	GCA	GCT	CAG	TAA	AACATAAAACCGCGCTTGGCCCCCGCGGTTTTTATTTTCTCTCCGCATGTTCAATCCGCTCC																	
1316	ATA	TCC	GAC	GAT	GGT	CCCTCTCTGAAATTTTAA	CAGAGAAACGGGGGTTGACCCCGCTCAGTCCCGTAACGGCCAGTCTGAAACGTCTCAATCCGCCG																		
1416	CTT	CCG	GTTTCCGGT	CAGCTCAATGCCGTAACGGTGGGGGTTTTCTCTGATACCGGAGACGGCATTCGTAATCGGATC																					

FIG._1B - 3

FIG._1B - 1
FIG._1B - 2
FIG._1B - 3

FIG._1B

CONSERVED RESIDUES IN SUBTILISINS FROM
BACILLUS AMYLOLIQUEFACIENS

1 10 20
A Q S V P . G A P A . H . . G

21 30 40
. T G S . V K V A V . D . G H P

41 50 60
D L . . . G G A S . V P Q D

61 70 80
. N . H G T H V A G T . A A L N N S I G

81 90 100
V L G V A P S A . L Y A V K V L G A . G

101 110 120
S G . . . S . L . . . G . E W A . N

121 130 140
V . N . S L G . P S . S A . .

141 150 160
. G V . V V A A . G N . G . . .

161 170 180
. Y P . . Y A V G A .

181 190 200
D . . N . . A S F S . . G . . L D . . A

201 210 220
P G V . . Q S T . P G . . Y N G T

221 230 240
S M A . P H V A G A A A L K . . .

241 250 260
W . . . Q . R . . L . N T . . . L G . .

261 270
. . Y G . G L . N . . A A . .

FIG. 2

0906087-04598

COMPARISON OF SUBTILISIN SEQUENCES FROM:

B. amyloliquefaciens
B. subtilis
B. licheniformis
B. lentus

01	10	20	30	
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P				
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P				
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P				
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T * H P				
41	50	60	70	
D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G				
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G				
D L N V R G G A S F V A G E A Y N * T D G N G H G T H V A G T V A A L N N T T G				
D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G				
81	90	100	110	
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D				
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D				
V L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T T N G M D				
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A C N N G M H				
121	130	140	150	
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V A A A A G N E G T S G				
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V A A A A A G N E G S S G				
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V A A A A G N S G N S G				
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S				

FIG._3A

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161      170      180      190
SSSTVGYPPGKYPSSVIAVGAVDSSNQRASSFSSVGP ELDVMA
STSTVGYPPAKYPPSTIAVGA VNSSNQRASSFSSAGSELDVMA
STNTIGYPPAKYDSSVIAVGA VDNSSNQRASSFSSAGSELDVMA
* * * I S Y P A R Y A M A V G A T D Q N N R A S F S Q Y G A G L D I V A

201      210      220      230
PGVSIQSTLPGGNKYGA YN G T S M A S P H V A G A A A L I L S K H P N
PGVSIQSTLPGGTKYGA YN G T S M A T P H V A G A A A L I L S K H P T
PGAGVYSTYPPNTYA T L N G T S M A S P H V A G A A A L I L S K H P N
PGVNVQSTYPPGSTYAS L N G T S M A T P H V A G A A A L V K Q K N P S

241      250      260      270
WTNTQVVRSSLENTT T K L G D S F Y Y G K G L I N V Q A A A Q
WTNAQQVVRRLLESSTAT T Y L G N S F Y Y G K G L I N V Q A A A Q
LSASQQVVRRLLESSTAT T Y L G S S F Y Y G K G L I N V E A A A Q
WSNVQI R R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R

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FIG._3B

FIG._3

FIG._3A

FIG._3B

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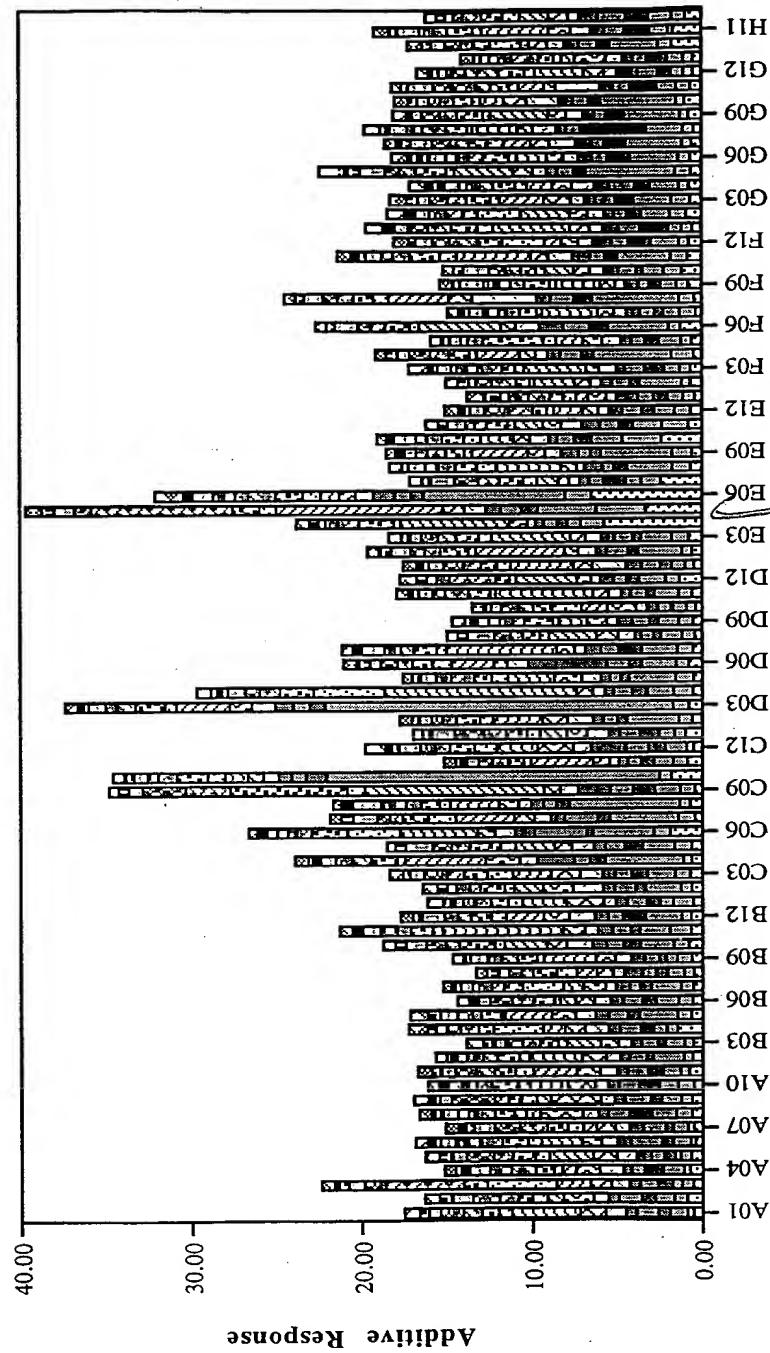


FIG. 4

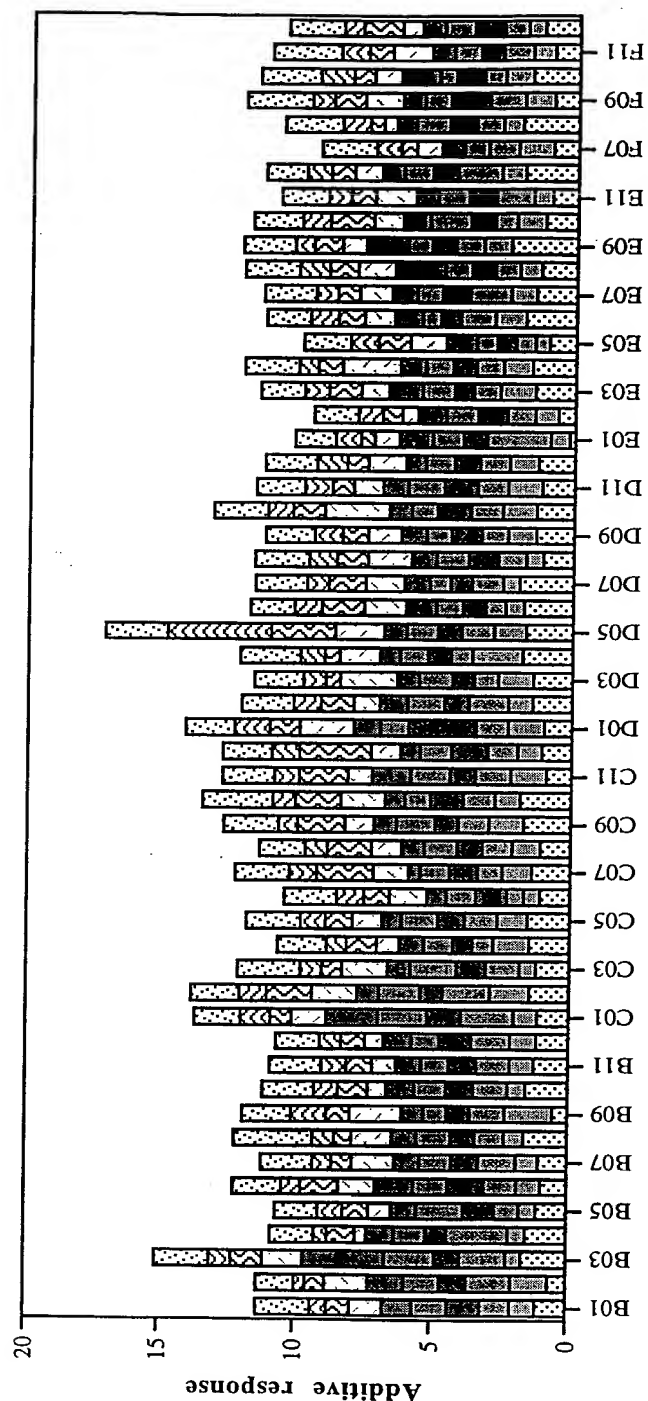


FIG. 5

1	A12	IKDFHVYFRESRDAG	49	E12	SATSRGVLVVAASGN
2	A11	LEQAVNSATSRGVLV	50	E11	SRGVLVVAASGNSGA
3	A10	AQSVPWGISRVQAPA	51	E10	VLVVAASGNSGAGSI
4	A9	VPWGISRVQAPAAHN	52	E9	VAASGNSGAGSISYP
5	A8	GISRVQAPAAHNRGL	53	E8	SGNSGAGSISYPARY
6	A7	RVQAPAAHNRGLTGS	54	E7	SGAGSISYPARYANA
7	A6	APAAHNRGLTGSGVK	55	E6	GSISYPARYANAMAV
8	A5	AHNRGLTGSGVKVAV	56	E5	SYPARYANAMAVGAT
9	A4	RGLTGSGVKVAVLDT	57	E4	ARYANAMAVGATDQN
10	A3	TGSGVKVAVLDTGIS	58	E3	ANAMAVGATDQNNNR
11	A2	GVKVAVLDTGISTHP	59	E2	MAVGATDQNNNRASF
12	A1	VAVLDTGISTHPDLN	60	E1	GATDQNNNRASFQY
13	B12	LDTGISTHPDLNIRG	61	F12	DQNNNRASFQYGAG
14	B11	GISTHPDLNIRGGAS	62	F11	NNRASFSQYGAGLDI
15	B10	THPDLNIRGGASFVP	63	F10	ASFSQYGAGLDIVAP
16	B9	DLNIRGGASFVPGEF	64	F9	SQYGAGLDIVAPGVN
17	B8	IRGGASFVPGEFSTQ	65	F8	GAGLDIVAPGVNVQS
18	B7	GASFVPGEFSTQDGN	66	F7	LDIVAPGVNVQSTYP
19	B6	FVPGEFSTQDGNHGH	67	F6	VAPGVNVQSTYPGST
20	B5	GEPSTQDGNHGHGTHV	68	F5	GVNVQSTYPGSTYAS
21	B4	STQDGNHGHGTHVAGT	69	F4	VQSTYPGSTYASLNG
22	B3	DGNHGHGTHVAGTIAA	70	F3	TYPGSTYASLNGTSM
23	B2	GHGTHVAGTIAALNN	71	F2	GSTYASLNGTSMATP
24	B1	THVAGTIAALNNSIG	72	F1	YASLNGTSMATPHVA
25	C12	AGTIAALNNSIGVLG	73	G12	LNGTSMATPHVAGAA
26	C11	IAALNNSIGVLGVAP	74	G11	TSMATPHVAGAAALV
27	C10	LNNSIGVLGVAPSAE	75	G10	ATPHVAGAAALVKQK
28	C9	SIGVLGVAPSAELYA	76	G9	HVAGAAALVKQKNPS
29	C8	VLGVAPSAELYAVKV	77	G8	GAAALVKQKNPSWSN
30	C7	VAPSAELYAVKVLGA	78	G7	ALVKQKNPSWSNVQI
31	C6	SAELYAVKVLGASGS	79	G6	KQKNPSWSNVQIRNH
32	C5	LYAVKVLGASGSGSV	80	G5	NPSWSNVQIRNHLKN
33	C4	VKVLGASGSGSVSSI	81	G4	WSNVQIRNHLKNTAT
34	C3	LGASGSGSVSSIAQG	82	G3	VQIRNHLKNTATSLG
35	C2	SGSGSVSSIAQGLEW	83	G2	RNHLKNTATSLGSTN
36	C1	GSVSSIAQGLEWAGN	84	G1	LKNTATSLGSTNLYG
37	D12	SSIAQGLEWAGNNGM	85	H12	TATSLGSTNLYGSGL
38	D11	AQGLEWAGNNGMHVA	86	H11	SLGSTNLYGSGLVNA
39	D10	LEWAGNNGMHVANLS	87	H10	STNLYGSGLVNAEAA
40	D9	AGNNGMHVANLSLGS	88	H9	NLYGSGLVNAEAATR
41	D8	NGMHVANLSLGSPSP			
42	D7	HVANLSLGSPSPSAT			
43	D6	NLSLGSPSPSATLEQ			
44	D5	LGSPSPSATLEQAVN			
45	D4	PSPSATLEQAVNSAT			
46	D3	SATLEQAVNSATSRG			
47	D2	LEQAVNSATSRGVLV			
48	D1	AVNSATSRGVLVVA			

FIG. 6A

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1	A12	IKDFHVFYFRESRDAG	49	E12	KKIDVLNLSIGGPDF
2	A11	DAELHIFRVFTNNQV	50	E11	DVLNLSIGGPDEMDH
3	A10	PLRRASLSLGS GFHW	51	E10	NLSIGGPDEMDHPFV
4	A9	RASLSLGS GFWHATG	52	E9	IGGPDEMDHPFVDKV
5	A8	LSLGS GFWHATGRHS	53	E8	PDFMDHPFVDK VWEL
6	A7	GSGFWHATGRHSSRR	54	E7	MDHPFVDK VWELTAN
7	A6	FWHATGRHSSRLLR	55	E6	PFVDK VWELTANNVI
8	A5	ATGRHSSRLLRAIP	56	E5	DKVWELTANNVIMVS
9	A4	RHSSRLLRAIPRQV	57	E4	WELTANNVIMVSAIG
10	A3	SRLLRAIPRQVAQT	58	E3	TANNVIMVSAIGNDG
11	A2	LLRAIPRQVAQTLQA	59	E2	NVIMVSAIGNDGPLY
12	A1	AIPRQVAQTLQADV L	60	E1	MVSAIGNDGPLYGTJ
13	B12	RQVAQTLQADV LQOM	61	F12	AIGNDGPLYGTLNPN
14	B11	AQTLQADV LQOMGYT	62	F11	NDGPLYGTLNPNADQ
15	B10	LQADV LQOMGYTGAN	63	F10	PLYGTLNPNADQMDV
16	B9	DVLWQOMGYTGANVRV	64	F9	GTLNPNADQMDVIGV
17	B8	WQOMGYTGANVRVAVF	65	F8	NNPADQMDVIGVGGI
18	B7	GYTGANVRVAVFDTG	66	F7	ADQMDVIGVG GIDFE
19	B6	GANVRVAVFDTGLSE	67	F6	MDVIGVG GIDFEDNI
20	B5	VRVAVFDTGLSEKHP	68	F5	IGVG GIDFEDNIARF
21	B4	AVFDTGLSEKHPHFK	69	F4	GGIDFEDNIARFSSR
22	B3	DTGLSEKHPHFKNVK	70	F3	DFEDNIARFSSRGMT
23	B2	LSEKHPHFKNVKERT	71	F2	DNIARFSSRGMTTWE
24	B1	KHPHFKNVKERTNWT	72	F1	ARFSSRGMTTWELPG
25	C12	HFKNVKERTNWTNER	73	G12	SSRGMTTWELPGGYG
26	C11	NVKERTNWTNERTLD	74	G11	GMTTWELPGGYGRMK
27	C10	ERTNWTNERTLDDGL	75	G10	TWELPGGYGRMKPDI
28	C9	NWTNERTLDDGLGHG	76	G9	LPGGYGRMKPDIVTY
29	C8	NERTLDDGLGHGTFV	77	G8	GYGRMKPDIVTYGAG
30	C7	TLDDGLGHGTFVAGV	78	G7	RMKPDIVTYGAGVRG
31	C6	DGLGHGTFVAGVIAS	79	G6	PDIVTYGAGVRGSGV
32	C5	GHGTFVAGVIASMRE	80	G5	VTYGAGVRGSGVKGG
33	C4	TFVAGVIASMRECQG	81	G4	GAGVRGSGVKGGCRA
34	C3	AGVIASMRECQGFAP	82	G3	VRGSGVKGGCRALSG
35	C2	IASMRECQGFAPDAE	83	G2	SGVKGGCRALSGTSV
36	C1	MRECQGFAPDAELHI	84	G1	KGGCRALSGTSVASP
37	D12	CQGFAPDAELHIFRV	85	H12	CRALSGTSVASPVVA
38	D11	FAPDAELHIFRVFTN	86	H11	LSGTSVASPVVAGAV
39	D10	DAELHIFRVFTNNQV	87	H10	TSVASPVVAGAVTLL
40	D9	LHIFRVFTNNQVSYT	88	H9	ASPVVAGAVTLLVST
41	D8	FRVFTNNQVSYTSWF	89	H8	VVAGAVTLLVSTVQK
42	D7	FTNNQVSYTSWFLDA	90	H7	GAVTLLVSTVQKREL
43	D6	NQVSYTSWFLDAFNY	91	H6	TLLVSTVQKRELVNP
44	D5	SYTSWFLDAFNYAIL	92	H5	VSTVQKRELVNPASM
45	D4	SWFLDAFNYAILKKI	93	H4	VQKRELVNPASMKQA
46	D3	LDAFNYAILKKIDVL	94	H3	RELVNPASMKQALIA
47	D2	FNAILKKIDVLNLS	95	H2	VNPASMKQALIASAR
48	D1	AILKKIDVLNLSIGG	96	H1	ASMKQALIASARRLP

FIG. 6B

12/16

97	I12	IKDFHVYFRESRDAG
98	I11	DAELHIFRVFTNNQV
99	I10	KQALIASARRLPGVN
100	I9	LIASARRLPGVNMFE
101	I8	SARRLPGVNMFEQGH
102	I7	RLPGVNMFEQGHGKL
103	I6	GVNMFEQGHGKLDLL
104	I5	MFEQGHGKLDLLRAY
105	I4	QGHGKLDLLRAYQIL
106	I3	GKLDLLRAYQILNSY
107	I2	DLLRAYQILNSYKPQ
108	I1	RAYQILNSYKPQASL
109	J12	QILNSYKPQASLSPS
110	J11	NSYKPQASLSPSYID
111	J10	KPQASLSPSYIDLTE
112	J9	ASLSPSYIDLTECPY
113	J8	SPSYIDLTECPYMWP
114	J7	YIDLTECPYMWPYCS
115	J6	LTECPYMWPYCSQPI
116	J5	CPYMWPYCSQPIYYG

FIG. 6C

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MKLVNIWLLLLLVLLCGKKHLGDRLEKKSFEKAPCPGCSHLTLKVEFSSTVVEYEYIVAFNGYFT
AKARNSFISSALKSSEVDNWRIIPRNNPSSDYPSEFEVIQIKEKQKAGLLTLEDHPNIKRVTQQR
KVFRSLKYAESDPTVPCNETRWSQKWQSSRPLRRASLSLGS GFWHATGRHSSRLLRAIPRQVAQ
TLQADVLWQMGYTGANVRVAVFDTGLSEKHPHFKNVKERTNWTNERTLDDGLGHGTFVAGVIASM
RECQGFAPDAELHIFRVFTNNQVSYTSWFLDAFNAILKKIDVLNLSIGGPDFMDHPFVDKVWEL
TANNVIMVSAIGNDGPLYGTLNPNADQMDVIGVGGIDFEDNIARFSSRGMTTWELPGGYGRMKPD
IVTYGAGVRGSGVKGGCRALSGTSVASPVVAGAVTLLVSTVQKRELVPASMKQALIASARRLPG
VNMFEQGHGKLDLLRAYQILNSYKQASLSPSYIDLTECPYMWPYCSQPIYYGGMPTVVNVNVTILN
GMGVTGRIVDKPDWQPYLPQNGDNIEVAFSYSSVLWPWSGYLAISISVTKKAASWEGIAQGHVMI
TVASPAETESKNGAEQTSTVKLPIKVKIIPTPPRSKRVLWDQYHNLRYPFGYFPRDNLRMKNDPL
DWNGDHIHTNFRDMYQHLRSMGYFVEVLGAPFTCFDASQYGTLLMVDSEEEYFPEEIAKLRRDVD
NGLSLVIFSDWYNTSVMRKVKFYDENTRQWWMPDTGGANIPALNELLSVWNMGFSDGLYEGETL
ANHDMYYASGCSIAKFPEDGVVITQTFKDQGLEVLKQETAVVENVPILGLYQIPAEAGGGRIVLYG
DSNCLDDSHRQKDCFWLLDALLQYTSYGVTPPSLSHSGNRQRPPSGAGSVTPERMEGNHLHRYSK
VLEAHLGDPKPRPLPACPRLSWAKPQPLNETAPSNLWKHKQLLSIDLKVVLPNFRSNRPQVRPL
SPGESGAWDIPGGIMPGRYNQEVGQTI PVFAFLGAMVVLAFVQINKAKSRPKRRKPRVKRPQL
MQQVHPPKTPSV

FIG. 7

	10	20	30	40	50	
BPN'	A Q S V P Y G V S Q - I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P D L K - V A G G A					48
SAVINASE	A Q S V P W G I S R - V Q A P A A H N R G L T G S G V K V A V L D T G I - S T H P D L N - I R G G A					47
S2HSBT	- R A I P R Q V A Q T L Q A D V L W Q M G Y T G A N V R V A V F D T G L S E K H P H F K N V K E R T					49
	60	70	80	90	100	
BPN'	S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G V L G V A P S A S L Y A V K V L G A					98
SAVINASE	S F V P G E P S T - Q D G N G H G T H V A G T I A A L N N S I G V L G V A P S A E L Y A V K V L G A					96
S2HSBT	N W - - T N E R T L D D G L G H G T F V A G V I A S M R E C Q G F - - - A P D A E L H I F R V F T N					94
	110	120	130	140	150	
BPN'	D G S G Q Y S W I I N G I E W A I A N N M D V I N M S L G G P S - G S A A L K A A V D K A V A S G V					147
SAVINASE	S G S G S V S S I A Q G L E W A G N N G M H V A N L S L G S P S - P S A T L E Q A V N S A T S R G V					145
S2HSBT	N Q V S Y T S W F L D A F N Y A I L K K I D V L N L S I G G P D F M D H P F V D K V W E L T A N N V					144
	160	170	180	190	200	
BPN'	V V V A A A G N E G T S G S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L -					197
SAVINASE	L V V A A S G N S G A - - - - G S I S Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G L -					191
S2HSBT	I M V S A I G N D G P - - L Y G T L N N P A D Q M D V I G V G G I D F E D N I A R F S S R G M T T W					192
	210	220	230	240	250	
BPN'	- - - - - D V M A P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L					235
SAVINASE	- - - - - D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K					229
S2HSBT	E L P G G Y G R M K P D I V T Y G A G V R G S G V K G G C R A L S G T S V A S P V V A G A V T L L V					242
	260	270	280	290		
BPN'	S K H P N W T N T Q - - - V R S S L E N T T T K L G D S F Y Y G K G L I N V Q A A A Q					275
SAVINASE	Q K N P S W S N V Q - - - I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R					269
S2HSBT	S T V Q K R E L V N P A S M K Q A L I A S A R R L P G V N M F E Q G - - - - - H G K L					280

FIG. 8

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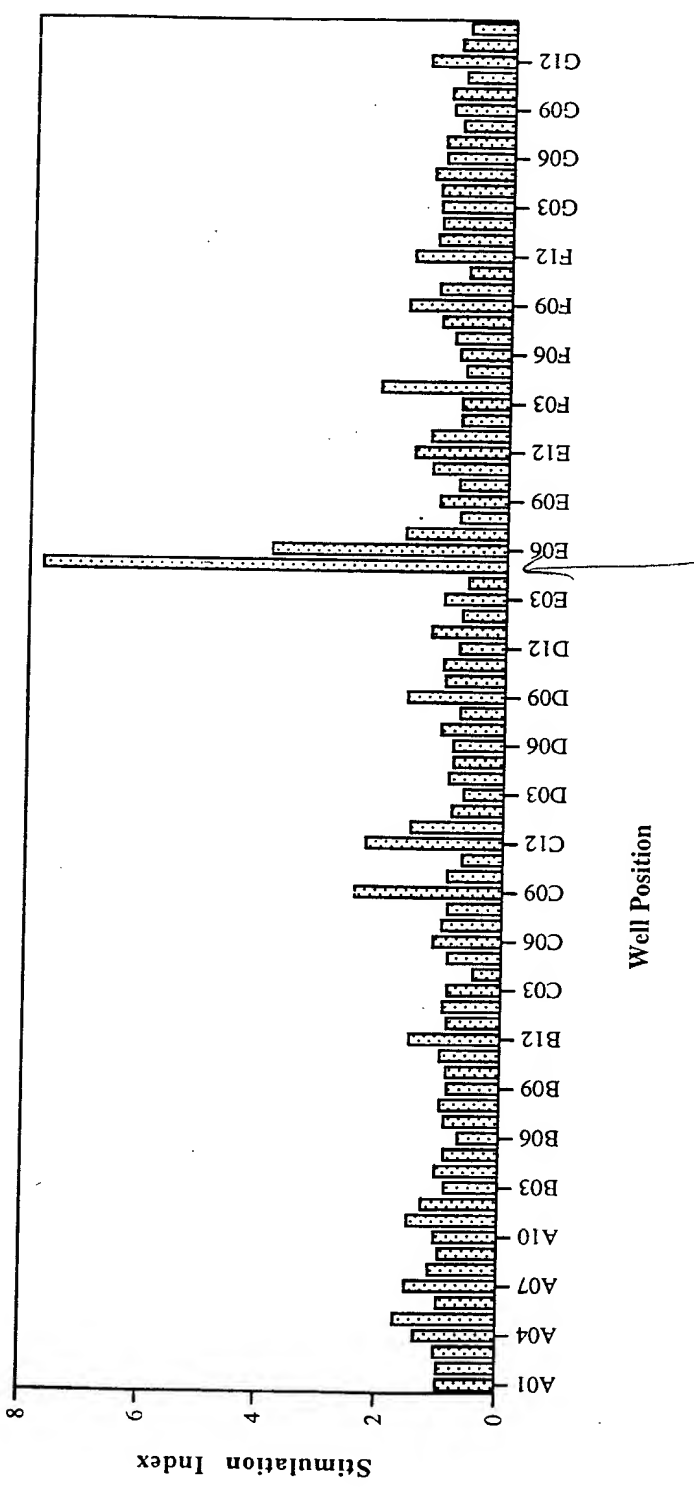


FIG. 9

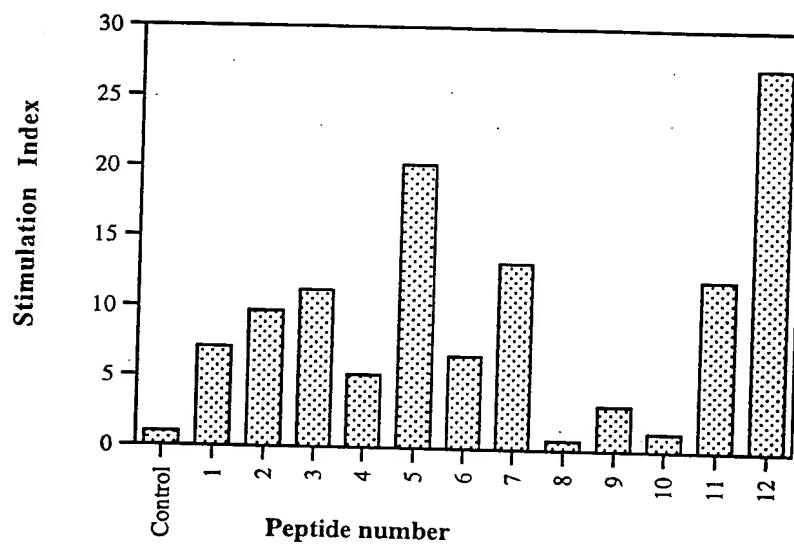


FIG. 10